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Research Article

Synthesis of hydroxyl silylated rhenium and (^{99m}Tc)technetium '3 + 1' mixed ligand complexes

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Summary

The synthesis of hydroxyl silylated thiols as monodentate ligands is described. These monodentates were used to build with Re and ^{99m}Tc trimethyl-, triethyl- and triphenyl-silylated '3 + 1' mixed ligand complexes, using 3thiapentane-1,5-dithiol as co-ligand. The Re complexes were characterized by ^{1}H NMR and elemental analysis, the ^{99m}Tc complexes were detected by radio HPLC. While the trimethyl silylated derivatives hydrolysed in aqueous media, the triethyl- and triphenyl silylated complexes have proved to be stable in neutral solutions.

Key Words: hydroxyl silylated thiols; Re and ^{99m}Tc complexes

Introduction

^{99m}Tc and ^{186}Re radiopharmaceuticals are widely used for diagnosis and therapy in nuclear medicine. Their transportation and accumulation *in vivo* is a crucial step but often unclear and many potential applications suffer from insufficient concentration of the radioactive drug in the target organ. Since membrane transport and accumulation

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of compounds in any organ system are governed by basic molecular parameters such as molecular size, charge, lipophilicity, etc., the availability of principles that allow one to control such parameters in vivo are valuable tools in studying bio-distribution patterns.

A promising way to increase the lipophilicity of organic compounds is the introduction of silyl groups, and several studies have shown that silylation of biologically active molecules facilitates their transportation in the organism.[1–4] By applying this principle of silylation to Re and ^{99m}Tc complexes, their biological properties should be improved and positive physiological effects are expected. Two major aims should be pursued by introduction of silylated groups. First, silylation of hydroxyl moieties usually increases the lipophilicity of the complex compared with the non-silylated one. This should improve the bio-availability as well, especially in terms of passing the blood brain barrier and the uptake of the compound in brain tissues. Second, in case of silyl ethers, which are hydrolyzable in vivo, the silylated radiopharmaceutical would be able to act as a pro-drug, because after cleavage of the labile silyl group the free hydroxyl compound should be trapped in the brain.

In previous work, the authors have described the synthesis of silylated '3 + 1' rhenium(V)oxo-complexes by silylation of the free hydroxyl side chain of hydroxylalkylthiolato(3thiapentane-1,5-dithiolato)oxo-rhenium(V) compounds.[5,6] The lipophilicity of the silylated Re complexes was determined[7] and their psychotropic activity and acute toxicity in vivo have been investigated[8]. However, the above synthetic pathway is not practicable for labelling with ^{99m}Tc and the silylated monodentate ligands should be prepared beforehand.

This paper reports the synthesis of trimethyl-, triethyl- and triphenyl-silylated alcohols bearing a free mercapto group and reports also the reaction of these monodentate ligands with chloro(3thiapentane-1,5-dithiolato)oxorhenium(V), an appropriate rhenium precursor in non-aqueous medium. We also investigate whether the silylated mercapto alcohols were stable against hydrolysis and suitable to form silylated '3 + 1' mixed ligand ^{99m}Tc complexes in aqueous solution.

Experimental

Materials and methods

All chemicals were of reagent grade and used without further purification. 2-Mercapto-ethanol, 3-mercapto-1-propanol, chloro-tri-

methylsilane, chloro-triethylsilane, chloro-triphenylsilane and imidazole were obtained from ALDRICH. The precursor chloro(3thiapentane-1,5-dithiolato)-oxorhenium(V) was prepared according to the literature[9]. Stannous chloride and sodium gluconate were purchased from ALDRICH. (^{99m}Tc)TcO_4^- in saline solution was eluted from a ^{99}Mo/^{99m}Tc generator from MDS Nordion S.A., Belgium.

Melting points were determined on a digital melting point apparatus IA 9200, Electrothermal, UK. Elemental analysis was performed on a Perkin-Elmer automatic analyser. The ^{1}H NMR spectra were recorded on a VARIAN Inova 300 MHz spectrometer. ^{1}H chemical shifts were referenced with the residual solvent resonance relative to tetramethylsilane. The NMR samples were prepared in $CDCl_3$.

HPLC investigations were carried out with an RP 18 column (Nova-Pack, 3.9 × 150 mm, Waters, USA) using a gradient of methanol/water as eluent with a flow rate of 1.0 ml/min. The products were determined by UV absorbance at 254 nm with a UV-VIS detector LC 290 (Perkin-Elmer) and by γ-detection with a scintillation detector LB 507A (Berthold).

O-alkylsiloxy-ethan-2thiols and -propan-3thiols 1a–e (general procedure)

2.0 mmol of mercaptoalcohol and 2.0 mmol imidazole (Imz) were stirred in 3.0 ml of dry DMF under a nitrogen atmosphere. 2.0 mmol of the chloro-trialkylsilane dissolved in 1.0 ml DMF was added and the mixture stirred for 24 h at room temperature. The DMF was removed by lyophilization under high vacuum and the residue was purified by column chromatography using short columns with silicagel and chloroform/hexane = 1:1 as eluent. In case of **1a**, **1c**, and **1d** the reaction was carried out without DMF using the mercapto alcohol as solvent and adding the pure chloro-trialkylsilanes.

1-Trimethylsiloxy-ethane-2thiol **1a**; liquid, yield 68%: ^{1}H NMR (δ, ppm): 0.12 (s, 9 H, $(CH_3)_3Si$), 1.49 (tr, 1 H, SH), 2,63 (q, 2 H, CH_2–S); 3.68 (tr, 2 H, CH_2–O).

1-Triphenylsiloxy-ethane-2thiol **1b**; m.p. 82–84°C, yield 65%: ^{1}H NMR (δ, ppm): 1.56 (tr, 1 H, SH), 2.68 (q, 2 H, CH_2–S), 3.91 (tr, 2 H, CH_2–O), 7.37–7.65 (m, 15 H, Ph). Elemental analysis: $C_{20}H_{20}OSSi$, theory C 71.42, H 5.95, S 9.52, found C 71.89, H 6.40, S 9.26.

1-Trimethylsiloxy-propane-3thiol **1c**; liquid, yield 49%: ^{1}H NMR (δ, ppm): 0.10 (s, 9 H, $(CH_3)_3Si$), 1.33 (tr, 1 H, SH), 1.81 (m, 2 H, CH_2), 2.61 (q, 2 H, CH_2–S), 3.67 (tr, 2 H, CH_2–O).

1-Triethylsiloxy-propane-3thiol **1d**; liquid, yield 72%: ^{1}H NMR (δ, ppm): 0.61 (q, 6 H, CH_2–Si), 0.95 (tr, 9 H, CH_3), 1.33 (tr, 1 H, SH), 1.81 (m, 2 H, CH_2), 2.63 (q, 2 H, CH_2–S), 3.70 (tr, 2 H, CH_2–O).

1-Triphenylsiloxy-propane-3thiol **1e**; m.p. 42–46°c, yield 68%: ^{1}H NMR (δ, ppm): 1.25 (tr, 1 H, SH), 1.86 (m, 2 H, CH_2), 2.66 (q, 2 H, CH_2–S), 3.89 (tr, 2 H, CH_2–O), 7.36–7.63 (m, 15 H, Ph). Elemental analysis: $C_{21}H_{22}OSSi$, theory C 72.00, H 6.28, S 9.14, found C 72.62, H 6.71, S 8.65.

(O-alkylsiloxy-alkylthiolato)(3thiapentane-1,5-dithiolato)oxo-rhenium(V) -complexes ***2a–e****:(general procedure):*

To 100 μmol of chloro(3thiapentane-1,5-dithiolato)oxorhenium(V) dissolved in 5.0 ml acetonitrile was added under stirring 25 μl (350 μmol) triethylamine followed by 200 μmol of the thiol **1** in 2.0 ml acetonitrile. The stirring was continued for 4 h at room temperature and then the solvent was evaporated off. The complexes were purified by column chromatography.

2a: Column chromatography: Silicagel, ethyl acetate, m.p. 126–129°C, yield 71%, ^{1}H NMR (δ, ppm): 0.15 (s, 9 H, $(CH_3)_3$Si), 1.97, 3.11, 3.90, 4.26 (m, 4 × 2 H, S-$(CH_2)_2$–S–$(CH_2)_2$–S), 3.97 (m, 2 H, CH_2–S), 4.01 (m, 2 H, CH_2–O). Elemental analysis: $C_9H_{21}O_2S_4SiRe$, theory, C 21.47, H 4.17, S 25.44, found, C 22.14, H 3.34, S 26.00.

2b: Column chromatography: Silicagel, chloroform/hexane 9:1, m.p. 163–167°C, yield 75%, ^{1}H NMR (δ, ppm): 1.91, 3.04, 3.86, 4.23 (m, 4 × 2 H, S–$(CH_2)_2$–S–$(CH_2)_2$–S), 4.08 (m, 2 H, CH_2–S), 4.16 (m, 2 H, CH_2–O) 7.34–7.68 (m, 15 H, Ph). Elemental analysis: $C_{24}H_{27}O_2S_4SiRe$, theory, C 41,79, H 5.92, S, 18.58, found, C 42.36, H 3.62, S 17.73.

2c: Column chromatography: silicagel, ethyl acetate, m.p. 92–96°C, yield 77%, ^{1}H NMR (δ, ppm): 0.13 (s, 9 H, $(CH_3)_3$Si), 1.94, 3.10, 3.78, 4.31 (m, 4 × 2 H, S-$(CH_2)_2$–S–$(CH_2)_2$–S), 2.15 (m, 2 H, CH_2), 3.88 (m, 2 H, CH_2–S), 3.93 (m, 2 H, CH_2–O). Elemental analysis: $C_{10}H_{23}O_2S_4SiRe$, theory, C 23.12, H 4.44, S 24.75, found, C 22.05, H 3.35, S 26.01.

2d: Column chromatography: silicagel, chloroform, m.p. 76–80°C, yield 80%, ^{1}H NMR (δ, ppm): 0.62 (q, 6 H, CH_2–Si), 0.96 (tr, 9 H, CH_3), 1.94, 3.10, 3.82, 4.29 (m, 4 × 2 H, S–$(CH_2)_2$–S–$(CH_2)_2$–S), 2.12 (m, 2 H, CH_2), 3.87 (m, 2 H, CH_2–S), 3.91 (m, 2 H, CH_2–O). Elemental analysis: $C_{13}H_{29}O_2S_4SiRe$, theory, C 27.90, H 5.18, S 23.02, found, C 26.42, H 4.58, S 23.28.

2e: column chromatography: Silicagel, chloroform/hexane 95:5, m.p.164–166°C, yield 74%, ^{1}H NMR (δ, ppm): 1.94, 3.10, 3.78, 4.31 (m, 4 × 2 H, S–$(CH_2)_2$–S–$(CH_2)_2$–S), 2.15 (m, 2 H, CH_2), 3.88 (m, 2 H, CH_2–S), 3.93 (m, 2 H, CH_2–O), 7.34–7.66 (m, 15 H, Ph). Elemental analysis: $C_{25}H_{29}O_2S_4SiRe$, theory, C 42.67, H 4.12, S 18,20, found, C 42.88, H 3.47, S 18.28

*(^{99m}Tc)O-alkylsiloxy-alkylthiolato)(3thiapentane-1,5-dithiolato)-oxo-technetium(V) complexes **4** (general procedure)*

To 500 µl of sodium gluconate (0.1 M) was added 500 µl of $^{99m}TcO_4^-$ (50–90 MBq) and the pertechnetate reduced using 10 µl of stannous chloride in 0.1 M HCl (0.01 M). The complete reduction of the pertechnetate to (^{99m}Tc)technetium gluconate was checked by thin layer chromatography (silicagel, acetone). The pH of the solution was adjusted to 7.1 by adding 500 µl of phosphate buffer (pH = 7.54). Afterwards, 1.5 ml of acetonitrile was added, followed by 2.0 µmol of the monodentate ligand **1** dissolved in acetonitrile. The closed vial was heated for 15 minutes at 50°C, cooled and then 0.5 µmol of the 3thiapentane-1,5-dithiol in acetonitrile was added. The mixture was heated for 15 minutes, at 50°C, and analysed by HPLC, using an RP 18 column and a flow rate of 1.0 ml/min of methanol/water: Gradient:10 - min 50% methanol ''90% methanol, 5 min 90% methanol isokratic, 5 min 90% methanol'' 50% methanol. The radiochemical yield of the (^{99m}Tc) complexes were determined from the radiochromatograms: **4b** yield 88%, **4d** yield 80%, **4e** yield 84%

Results and discussion

The synthetic pathway to obtain silylated '3 + 1' mixed ligand complexes of Re and ^{99m}Tc is outlined in Figure 1. The first step was the preparation of silylated mercapto alcohols by silylation of the free hydroxyl group and keeping the free mercapto functionality. These O-silylated mercapto alcohols are not only required for following the synthetic route, they are also a prerequisite for labelling with (^{99m}Tc) technetium.

Up to now the silylation of mercapto alcohols has not been referred to in detail[5,6] but it is known that in the reaction of chlorosilanes with alcohols in dimethylformamide, imidazole acts as a nucleophilic

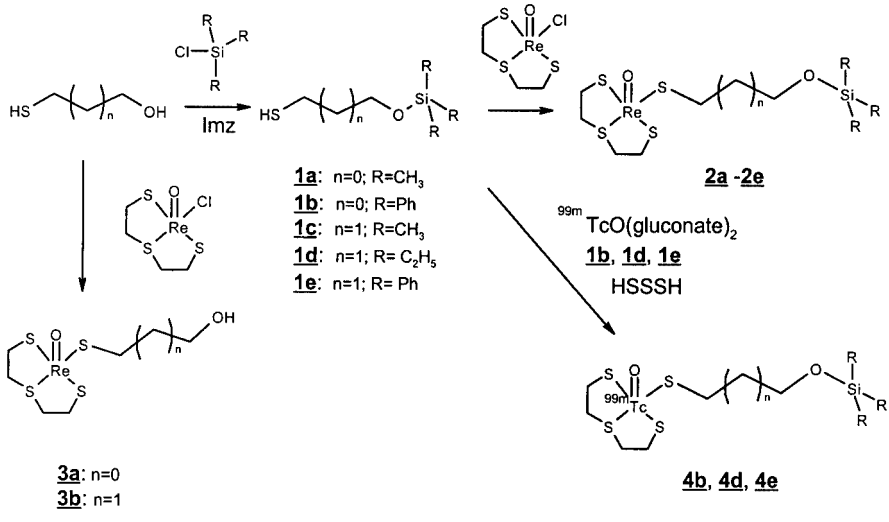

Figure 1. Synthetic pathway for silylated Re and ^{99m}Tc '3 + 1' mixed ligand complexes

catalyst[10]. By stirring 1,2-mercaptoethanol and 1,3-mercaptopropanol with the corresponding chlorosilanes under the above conditions at room temperature we found indeed a high rate of O-silylation and the mercapto group has not been attacked. In case of compounds **1a**, **1c** and **1d** the silylation was carried out in the absence of any solvent.

The silylated Re(V)oxo-complexes **2a–e** were prepared by reacting the free mercapto group of the silylated monodentates **1a–e** with the appropriate rhenium precursor chloro(3thiapentane-1,5-dithiolato)oxorhenium(V)[9] (Figure 1). A slight excess of triethylamine is necessary to avoid hydrolysis of the silyl group by the hydrochloric acid formed during the reaction. The triethyl and triphenyl silylated complexes were found to be stable against hydrolysis during the work up. In contrast, in the trimethyl-silylated complexes **2a** and **2c** traces of the hydrolysed by-products were still found, and the compounds hydrolysed completely by treatment with protonic solvents. The hydrolysed complexes **3a** and **3b**, were synthesised as previously described, and used as a reference (Figure 1)[6].

The preparation of the silylated (^{99m}Tc)(3-thiapentane-1,5-dithiolato)oxo-technetium(V) complexes **4** was performed via ^{99m}Tc gluconate in aqueous solution (Figure 1). After reduction of $^{99m}TcO_4^-$ with stannous chloride, the formation of ^{99m}Tc gluconate was checked by HPLC (Figure 2). For preparing **4b**, **4d** and **4e** the pH was adjusted to

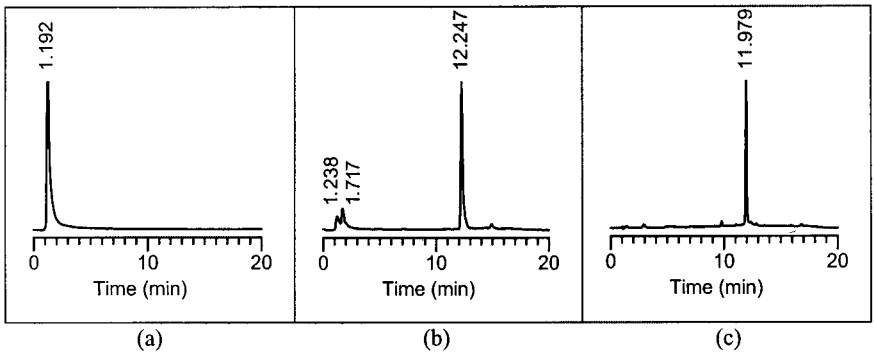

Figure 2. (a) HPLC radio chromatogram of ^{99m}Tc gluconate; (b) HPLC radio chromatogram of ^{99m}Tc complex 4b; (c) HPLC UV-VIS chromatogram of rhenium complex 2b

about 7 with phosphate buffer to avoid hydrolysis. The labelling was a two-step procedure: first the monodentate ligand **1** was added to the ^{99m}Tc gluconate solution and then the mixture was heated at 50°C. In the HPLC the ^{99m}Tc gluconate signal disappeared, but the intermediate could not be detected because it was too lipophilic. Secondly, the 3thiapentane-1,5-dithiol (HSSSH) was added followed by additional heating. The nature of the silylated '3 + 1' ^{99m}Tc complexes **4** were established by HPLC, by comparison with the analogous rhenium compounds, fully characterized at the macroscopic level (Figure 2).

For the triphenyl- and triethyl-silylated (^{99m}Tc)technetium(V)oxo-complexes **4b**, **4d** and **4e** labelling yields between 80 and 90% were found, together with some unreacted ^{99m}Tc gluconate. HPLC studies (comparison of retention times with those of the rhenium complexes **3a** and **3b**) showed that no hydrolysed ^{99m}Tc compounds were formed, thereby confirming their stability under the experimental conditions.

In the case of the trimethyl-silylated ligands **1a** and **1c** the labelling was unsuccessful. All the attempts made, led to a mixture of products containing the hydrolysed complexes.

Concluding remarks

'3 + 1' mixed ligand complexes of Re and ^{99m}Tc with O-silylated mercapto alcohols as monodentate ligands and 3-thia-pentane-1,5-dithiol as tridentate ligand have been synthesized. The new ligands and

the rhenium complexes were characterized by ^{1}H NMR and elemental analysis. The preparation of ^{99m}Tc complexes was carried out via ^{99m}Tc gluconate and their purity evaluated by HPLC. The yields were found to be 80–90% for the triethyl- and triphenyl-silylated complexes. These compounds are stable in aqueous conditions (pH = 7), in contrast to the trimethyl-silylated complexes which are unstable and hydrolyse in protic solvents. The silylated ^{99m}Tc complexes **4b**, **4d** and **4e** are promising for further biological investigation.

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